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Identification of genome-linked bacillus bacteriophage proteins

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IDENTIFICATION OF GENOME-LINKED BACILLUS BACTERIOPHAGE PROTEINS

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Abstract

Small viruses that can infect bacteria, called bacteriophages, can be found in the soil. These viruses are being examined as a potential treatment against bacterial infections when antibiotics are not available or non-effective. Before viruses can be used as a medical treatment, they must be studied extensively. Previous attempts to sequence the terminal end of the genome of a bacteriophage QCM-11 (Quartz-Crystal-Microbalance-11: Q11) resulted in error. It is thought that a covalently bound protein may be attached to Q11's DNA, blocking sequencing of the genome from the 5' end. Proteins bound to the DNA of phages is not unheard of, a well-documented phage, Phi29, has a protein bound to the 5' terminal end of its DNA. Both phages were studied to determine if Q11 has a terminally bound protein similar to Phi29. Phages Phi29 and Q11 were subjected to digestion by various restriction enzymes as well as a 5' exonuclease. If a protein is bound to the DNA of Q11 digestion by these nucleases would be blocked. The remaining fragments of phage DNA were run on agarose and polyacrylamide gels. The results from the experiment were not able to conclusively prove that a protein is bound to Q11 DNA.

Table of Contents

Introduction

Bacteria are among the most abundant organisms on the planet. Most bacteria are non-harmful and, in some cases, even essential for human life. They are also a source of infection that affects all living organisms. Antibiotics are the most common form of treatment to combat bacterial infections, but like all organisms, bacteria evolve and can become resistant to medical treatment over time. A lack of other medical interventions to treat bacterial infections poses a major public health risk. As antibiotics rush to keep up with evolving microorganisms, other methods of combating bacterial infections need to be researched.

Like most species on the planet, bacteria have a natural predator. Viruses that infect bacteria, known as bacteriophages or phages, are more abundant in nature than bacteria and make up the largest percentage of host-parasite relationships on the planet. The overall research is being conducted on bacteriophages as a potential future source of treatment for bacterial infections.

Bacteriophage QCM-11 (Quartz-Crystal-Microbalance-11 - Q11) is thought to have a protein that is covalently bound to the end of its's DNA, similar to another small phage, Phi29. The function of these covalently bound proteins is mostly unknown. For Phi29, the 5' covalently bound protein is thought to aid in the packaging of DNA into the head of the virus.[2] Further exploration of the possible function of these genome-bound proteins will yield a better understanding of how these proteins assist in the survival or virulence of the bacteriophage. A better understanding about these species of bacteriophage, and terminally bound proteins in general, allows researchers to determine whether Phi29 or Q11 could be used as novel treatments for bacterial infections.

Purpose

Spores of the bacterial species *Bacillus anthracis* (*B. anthracis*), the causal agent of anthrax, can be found as long-lasting endospores in soil. Because of this, anthrax poses a constant danger of emergence in livestock and humans. Vaccinations against anthrax are not available to the general public, and antibiotics to treat the infection are most effective when given immediately after exposure.

Bacillus cereus (*B. cereus*) is a toxin-producing species of bacteria commonly associated with symptoms of food poisoning. Like *B. anthracis, B. cereus* has the ability to form spores of itself that allow it to survive harsh environmental conditions, and then repopulate in optimal conditions. *B. cereus'* widespread presence in the soil and resistance to basic decontamination methods mean, like most foodborne illnesses, that it can infect humans and livestock through contaminated food products. *B. cereus* has been increasingly linked to cases of non-gastrointestinal-tract infections, many of which have been serious or life-threatening.

The most widely used treatments for *B. cereus* and *B. anthracis* infections are antibiotics, which are not always effective. To help mitigate the health risks of *B. cereus* and *B. anthracis* infections in humans and animals, studies are being conducted on viruses that can infect and kill specific species of bacteria known as bacteriophages or phages. These phages are being studied as a potential supportive treatment, along with antibiotics to treat *Bacillus cereus and Bacillus anthracis* infections.

Phage Q11 has been identified as a bacteriophage that can infect *Bacillus cereus* and *Bacillus anthracis.* Previous attempts to determine the genetic sequence of Q11 have been unsuccessful. It is thought that a protein may be permanently bound to the end of Q11's DNA preventing it from being fully sequenced. This investigation aims to determine if a protein is

covalently (permanently) bound to the 5’ end of Q11’s DNA. Confirmation will allow further study into the function of this protein and its role in Q11’s virulence and survival in host bacteria.

Literature Review

Background

Phage Phi29 which infects *Bacillus subtilis* (*B. subtilis*) has been positively identified as having a 5’ protein covalently bound to the end of the phages linear DNA. [1, 2] Previous investigations into Phi29 have confirmed the existence of a 5’ terminal protein bound to the end of Phi29’s linear DNA.[1,2] These experiments utilized restriction endonucleases, or restriction enzymes, which cut phage DNA into smaller pieces. These pieces were subjected to treatment with 5’ and 3’ exonucleases which degrade phage DNA from both ends (5’ and 3’) of the DNA. The results of these experiments showed that there was a small section of DNA that could not be degraded with 5’ exonuclease.[2] Since this fragment of phage DNA could be degraded with 3’ exonuclease, the conclusion was made that something was bound to the phage DNA on the 5’ end that blocked the degradation of DNA by the 5’ exonuclease.

Assembly of the virus was done using radioactively labeled atoms. [^{3}H] thymidine, used for building DNA and [^{35}S] methionine, used for building proteins were used.[2] Another variation of this experiment used [^{14}C] thymidine and [^{14}C] methionine.[1] Viral proteins and DNA were separated out using sucrose gradient sedimentation. Most viral proteins were banded together on the gradient and were detected by measuring levels of radioactively labeled methionine. However, a small amount of radioactively labeled methionine was detected in the DNA band, suggesting some viral protein was bound to the phage DNA.[1,2] Treatment of the protein-bound DNA with various chemicals was not able to break the DNA-protein bond, suggesting the linkage was covalent in nature.[2]

Proteins covalently bound to the 5' end of small bacteriophage DNA are rare but not unheard of. Q11 is a small bacteriophage that can infect *Bacillus anthracis* Sterne, a safe non-pathogenic (vaccine) strain of *B. anthracis.* Previous attempts to sequence the 5' terminal sequence of the bacteriophage Q11 were unsuccessful.[3] The inability to sequence the end of Q11's DNA suggests there may be a similar covalently attached protein on the 5' end of Q11's DNA, similar to Phi29.[3,4,9] Like Phi29, a 5' terminal protein could prevent the complete DNA sequencing of Q11. Research completed by Emily Cornelius and Dr. Michael Walter in the summer of 2019 on Q11 was inconclusive on the existence of a covalently bound 5' terminal protein.[4,9]

Relevance

Bacillus cereus has been widely documented as a potential agent of food-borne illnesses causing gastrointestinal distress. Cases of *B. cereus* infections have also reported non-gastrointestinal symptoms that can be serious or life-threatening.[5, 6] *Bacillus anthracis* produces a toxin that is a known bio-terrorism agent.[7] Both of these bacterial species pose a risk to public health. The development of new antibiotics to treat bacterial infections has dramatically slowed down.[8] The World Health Organization (WHO) has also confirmed that bacteria are becoming increasingly resistant to antibiotics currently being used to treat infections, which is a major public health emergency.[8]

Materials and Methods

Bacteriophage DNA

A stock from bacteriophage Phi29 was purchased from the Sylvain Moineau, Universite Laval, in Quebec Canada. This stock of phage was grown up in *B. subtilis* strain 1046. Isolated DNA was purified by Emily Cornelius and Michael Walter on 2019-12-10 following standard

methodology.[10,11] Samples of purified phage DNA were stored in a freezer at -20 °C until use in further experiments. Phage DNA was stored on ice when in use.

Bacteriophage Q11 was isolated from planted prairie soil, enriched with *B. anthracis* by Michael Walter.[10] This phage was triple-serially-isolated for purity before being increased on plates of *B. cereus* before being harvested for phage DNA. This DNA was purified by ultracentrifugation by Emily Cornelius and Michael Walter, using standard methods[10], on 2019-06-18 before being stored in a freezer at -20 °C. Purified Q11 DNA was kept on ice while in use.

Enzyme Digestion of Phage DNA

Enzymatic digestion of bacteriophage DNA was a critical procedure for identifying terminally bound proteins on bacteriophage DNA. For endonuclease (restriction enzyme: RE) digestion, varying amounts of restriction enzyme, restriction enzyme buffer, bovine serum albumin (BSA) diluted in Promega Multi-core RE buffer, phage DNA and sterile distilled water were combined in microcentrifuge tubes. Restriction enzymes EcoRI 10 U/µL (ThermoFisher Scientific ER0271, 5,000 units) and HindIII 10 U/µL (ThermoFisher Scientific ER0501, 5,000 units) were used for DNA digestion. The buffers for EcoRI and HindIII were obtained from Promega Corporation (Madison WI) product numbers R008A and R005A, respectively. The BSA and RE buffers (Promega) served as stabilizing agents during enzyme digestion. Bacteriophage DNA used in these experiments was isolated from phage Phi29 and phage Q11 on 2019-12-10 and 2019-06-18, respectively by Emily Cornelius and Michael Walter at the University of Northern Iowa. Stocks of the bacteriophage DNA were used for enzyme digestion. Sterile distilled water was made in house by autoclaving glass-distilled water for one hour. All reagents and enzymes when not in use were stored in a freezer at -20 °C. While in use the reagents were kept on ice to preserve enzyme activity.

Exonuclease digestion of Phage DNA was completed using 5'-> 3' λ exonuclease (5' exonuclease) 10 U/ µL (ThermoFisher Scientific, EN0562, 5,000 units) following digestion by restriction enzyme EcoRI. This exonuclease was and stored in a freezer at -20 °C when not in use. While in use the 5' exonuclease was kept on ice to preserve enzyme activity.

Incubation of restriction enzymes and exonucleases was typically done in a water bath at 37 °C. Variations in temperature and incubation time are noted in the experimental results. After incubation, NEB Stop Buffer made in-house (50% glycerol, 50 mM EDTA (pH 8.0), 0.05% bromophenol blue (10 µl / 50 µl reaction) was added to the reaction to stop the activity of the enzyme at a ratio of 1 µL NEB Stop solution to 5 µL of reaction mixture.

Gel Electrophoresis

Purified phage DNA, phage DNA digested by restriction enzymes EcoRI and HindIII, and phage DNA digested by restriction enzyme EcoRI then digested again with 5' exonuclease was loaded onto gels for electrophoresis. The conditions listed in the methodology below is consistent for each experiment run unless noted in the results section.

Agarose

Agarose gels work like a porous sponge. Naturally negatively charged DNA fragments migrate through the holes of the sponge when an electric field is applied to them. Smaller fragments are more easily able to pass through the holes of the gel. The larger the DNA fragments are, the more tangled they get while passing through the gel and do not migrate as far down the gel as smaller fragments. This allows DNA fragments to be separated by size and shape.

Phi29 and Q11 DNA restriction enzyme and exonuclease digests were run on 0.8% agarose gels that were poured in the lab. The agarose for making the gels was obtained from ThermoFisher Scientific. The gels were run at 80 V for 60 minutes then 40V for four hours in a

0.5X concentration of Tris-Borate-EDTA running buffer (TBE, pH 8.3). TBE buffer was diluted from a stock 10X solution obtained from ThermoFisher Scientific (B52). Gels were stained with ethidium bromide (EtBr), at a final concentration of 0.2-0.5 µg/mL in 0.5X TBE for 20 minutes. After staining the gels were destained in TBE buffer then imaged on a UV lightbox using Carestream Imaging Technology (Carestream Health, Inc, Rochester, NY).

SDS PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) works similarly to agarose gels in that a purified sample is passed through a porous gel using an electric field. The fragments generally processed using SDS polyacrylamide are proteins because the holes in the polyacrylamide gel are too small for DNA that get stuck at the top of the gel. Proteins are denatured by boiling samples in Laemmli buffer (125 mM Tris-Cl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) for 3 minutes. This linearizes and simultaneously coats the proteins with sodium dodecyl sulfate (SDS). SDS imparts a negative charge and allows proteins to run through the SDS-PAGE gel, being separated by size, not charge or shape. Purified Q11 DNA and Q11 DNA treated with restriction enzyme (RE) EcoRI at varying concentrations – then were analyzed by SDS PAGE to reveal protein migration differences attributable to proteins attached to Q11 DNA. Treated DNA samples were loaded on and analyzed on 4–20% gradient Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 30 µl gels (Bio Rad, Hercules, CA, 4561093) and run in standard SDS-PAGE ‘Tris-Glycine’ running buffer (25 mM Tris, 193 mM glycine, 0.1% SDS). The gels were run at 150 V until markers reached the bottom of the gel, about 120 minutes.

After running, the proteins in the gel were treated with a fixative solution mixture of 40% ethanol and 10% acetic acid. The gels were rinsed twice with distilled water for 20 minutes. After rinsing the gels were stained with Coomassie Blue dye then extensively destained in the fixative solution. The gels were silver stained using reagents and methodology from Bio Rad Silver Stain

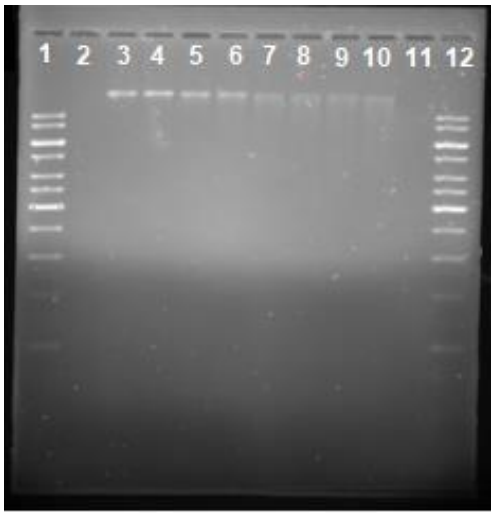
Plus Kit, (Bio Rad, Hercules, CA, 1610449) and imaged by transmitted light (not UV) as described above.

Results

The specific conditions for each experiment along with the results and gel images are discussed below. Each experiment is date coded in year-month-day format. For example, April 5th 2020 would be coded as 200405.

200110 EcoRI Restriction Enzyme Digestion of Phi29 DNA

Phi29 DNA isolated and purified on December 10th of 2019 (191210) was treated with restriction enzyme EcoRI and allowed to incubate for varying amounts of time to demonstrate the efficacy of RE EcoRI on Phi29 DNA



Lanes one and twelve on the agarose gel contain 5µL of 1kb *Generuler* DNA ladder (ThermoFisher) each, as a standard to measure the length of the restriction fragments. Lanes two and eleven on the gel were left blank. Lane 3 was loaded with 5 µL of Phi29 DNA, 2 µL of 10x EcoRI Buffer H, 2 µL of BSA diluted 10X in Promega Multi-core, 11 µL of sterile distilled water for a total volume of 20 µL loaded. Lanes four through ten were loaded with 20 µL each of a mixture containing 5 µL of Phi29 DNA, 2 µL of 10X EcoRI Buffer H, 2 µL of BSA diluted to 10X in Promega Multi-core, 2 µL of EcoRI restriction enzyme, and 9 µL of sterile distilled water. The contents of lanes three and four were not allowed to incubate, NEB Stop Buffer was added to the solution immediately after addition of the restriction enzyme. Tubes five, six, seven, eight, nine and ten were allowed to incubate for further enzyme digestion at 37 °C for one, two, three, six, eight, and ten minutes,

respectively, before NEB Stop Buffer was added to each tube. 20 µL of each mixture was added to lanes three through ten on an agarose gel.

The amount of phage DNA used in this digestion was insufficient, though the decrease in intensity of the phage DNA band is evident (Figure 1). The next digest of Phi29 DNA will use more phage DNA. The results of this experiment confirm that EcoRI is effective at digesting Phi29 DNA.

200127 EcoRI and HindIII Restriction Enzyme Digestion of Phi29 DNA

Phi29 DNA from 191210 was treated with restriction enzymes EcoRI and HindIII. Phi29 DNA digested with HindIII was allowed to incubate for varying amounts of time to confirm activity of the restriction enzyme. More phage DNA was used in this experiment after previous trials using 5 µL of phage DNA proved insufficient to visualize restriction fragments on an agarose gel.

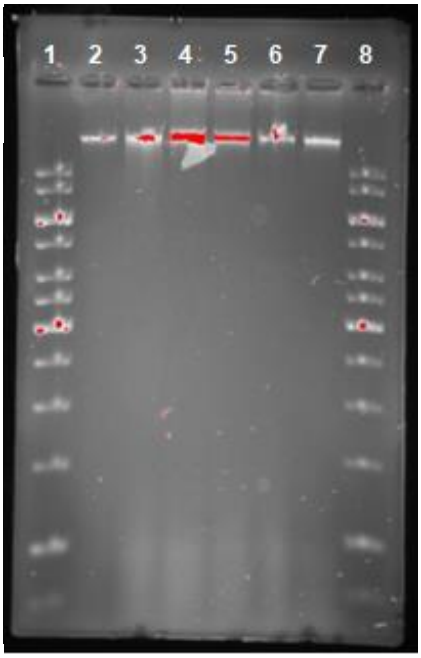


Figure 2. 200127 agarose gel of EcoRI and HindIII digestion of Phi29 DNA.

Lanes one and eight were loaded with 3 µL of 1kb *Generuler* DNA ladder. Lane two was loaded with a 30 µL aliquot containing 5 µL of Phi29 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted to 10X in Promega Multi-core, 19 µL of sterile distilled water and 6 µL of NEB Stop buffer. Lane three was loaded with a 30 µL aliquot containing 20 µL of Phi29 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted in Promega Multi-core, 2 µL of sterile distilled water, 2 µL of EcoRI restriction enzyme. The contents of lane 3 were allowed to incubate at 37 °C for 10 minutes before 6 µL of NEB Stop buffer was added. Lanes four, five, six and seven were each loaded with an aliquot containing 20 µL of Phi29 DNA, 3 µL of HindIII Buffer E, 3 µL of BSA diluted to 10X in Promega Multi-core, 2 µL of sterile distilled water and 2 µL of HindIII restriction enzyme. Each sample was allowed to incubate for ten, fifteen, twenty, and thirty minutes, respectively, before 6 µL of NEB Stop buffer was added to

each mixture. The agarose gel was run for one hour at 80V and then at 40V for five hours before being EtBr stained and imaged.

This experiment was a continuation from 200110 where 5 µL of Phi29 phage DNA subjected to EcoRI digestion was not enough to produce visible bands on an agarose gel. The results from this experiment (Figure 2) show that 20 µL of phage DNA produces undigested bands that are very bright on the gel. There was too much phage DNA, not enough restriction enzyme and not enough incubation time to digest the phage DNA to produce bands. Less phage DNA and a longer incubation time could be used to produce visible bands.

200130 EcoRI and HindIII Restriction Enzyme Digestion of Phi29 DNA

To determine the appropriate concentration of Phi29 DNA to load into the gel to produce visible bands after restriction enzyme digestion, another round of experiments similar to 200110 and 200127 was conducted. More Phi29 phage DNA was used than the 200110 experiment, but less than the amount of DNA used in the 200127 experiment.

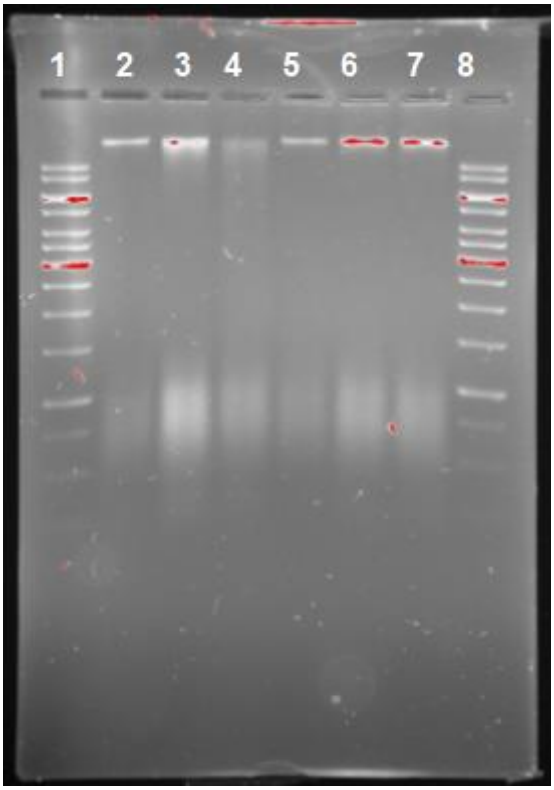


Figure 3. 200130 agarose gel of EcoRI and HindIII digestion of Phi29 DNA.

Lanes one and eight were loaded with 3 µL of 1 kb *Generuler* DNA ladder. Lane two was loaded with a 20 µL aliquot of a mixture containing 5 µL of Phi29 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted to 10x in Promega Multi-core, 9 µL of sterile distilled water and 4 µL of NEB Stop buffer. Lanes three and four were loaded with a 20 µL aliquot of a mixture containing 10 µL of Phi29 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted to 10x in Promega Multi-core, 2 µL of sterile distilled water and 2 µL of EcoRI restriction enzyme. Both tubes were incubated at 37 °C for 30 minutes before 4 µL of NEB Stop buffer was added to

the mixture. Lane five was loaded with a 20 µL aliquot of a mixture containing 5 µL of Phi29 DNA, 3 µL of HindIII buffer E, 3 µL of BSA diluted to 10x in Promega Multi-core, 9 µL of sterile distilled water and 4 µL of NEB Stop buffer. Lanes six and seven were loaded with a 20 µL aliquot of a mixture containing 10 µL of Phi29 DNA, 3 µL of HindIII buffer E, 3 µL of BSA diluted to 10x in Promega Multi-core, 2 µL of sterile distilled water and 2 µL of HindIII restriction enzyme. Both tubes were incubated at 37 °C for 30 minutes before 4 µL of NEB Stop buffer was added to the mixture. The agarose gel was run before being stained and imaged as described in the methods section above.

The amounts of Phi29 DNA loaded in this experiment produced visible DNA on the gel (Figure 3). However, digested Phi29 DNA fragments did not produce visible bands. Phi29 DNA purified on 191210 appears to contain contaminants that caused the DNA on the gel to smear. Phi29 is a well-documented bacteriophage for which enzyme restriction digests are known and predictable. The primary use of Phi29 was to set up the conditions for running restriction enzyme digests for Q11 DNA. The conditions for this experiment, in terms of concentration and incubation time of reagents will be used in future runs using Q11 DNA.

200203 EcoRI and HindIII Restriction Enzyme Digestion of Q11 DNA

Phage Q11 DNA was subjected to the same treatment as Phi 29 in the 200130 experiment. Phi29 DNA was used in previous experiments as a model to adjust for the right concentrations of DNA, enzyme and incubation time to yield fragments. Phi29 is a well-studied and documented bacteriophage whose results in our lab could be compared to previous work with Phi29.

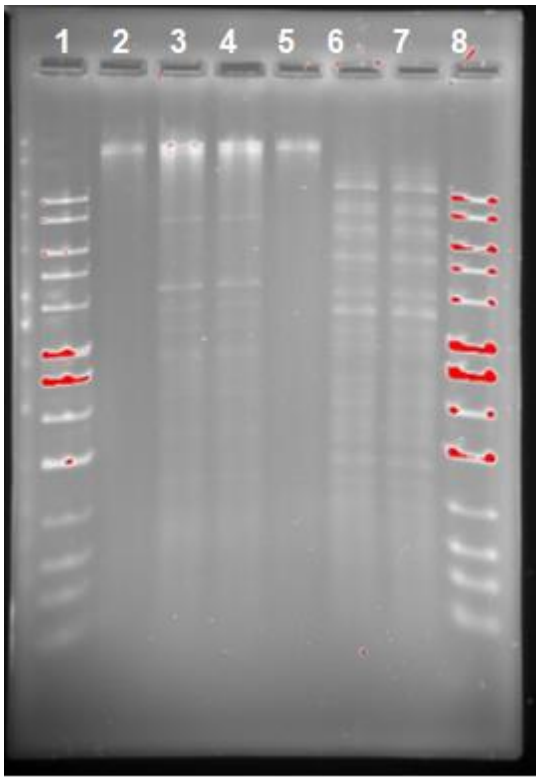


Figure 4. 200203 agarose gel of Q11 DNA digested with EcoRI and HindIII.

Lanes one and eight were loaded with 3 µL of 1 kb *Generuler* DNA ladder. Lane two was loaded with a 20 µL aliquot of a mixture containing 5 µL of Q11 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted to 10x in Promega Multi-core, 9 µL of sterile distilled water and 4 µL of NEB Stop buffer. Lanes three and four were loaded with a 20 µL aliquot of a mixture containing 10 µL of Q11 DNA, 3 µL of EcoRI buffer H, 3 µL of BSA diluted to 10x in Promega Multi-core, 2 µL of sterile distilled water and 2 µL of EcoRI restriction enzyme. Both tubes were incubated at 37 °C for 30 minutes before 4 µL of NEB Stop buffer was added to the mixture. Lane five was loaded with a 20 µL aliquot of a mixture containing 5 µL of Q11 DNA, 3 µL of HindIII buffer E, 3 µL of BSA diluted to 10x in Promega Multi-core, 9 µL of sterile distilled water and 4 µL of NEB Stop buffer. Lanes six and seven were loaded with a 20 µL aliquot of a mixture containing 10 µL of Q11 DNA, 3 µL of HindIII buffer E, 3 µL of BSA diluted to 10x in Promega Multi-core, 2 µL of sterile distilled water and 2 µL of HindIII restriction enzyme. Both tubes were incubated at 37 °C for 30 minutes before 4 µL of NEB Stop buffer was added to the mixture.

The restriction enzyme digestion of Q11 DNA using EcoRI and HindIII was successful (Figure 4). EcoRI digestion of Q11 yielded 9 visible fragments when run on agarose gel. HindIII

digestion of Q11 DNA yielded 17 visible fragments on agarose gel. These results are consistent with previous digestions of Q11 DNA performed by Emily Cornelius and Michael Walter in the summer of 2019.

200206 Large Scale EcoRI Restriction Enzyme Digestion of Q11 DNA

Q11 DNA digestion by EcoRI in the previous experiment, 200203, successfully produced visible bands on agarose gel. To continue experiments looking for a terminal protein bound, large amounts of restriction digested Q11 are needed. In this experiment larger amounts of Q11 DNA were subjected to EcoRI restriction enzyme digestion to produce Q11 DNA fragments.

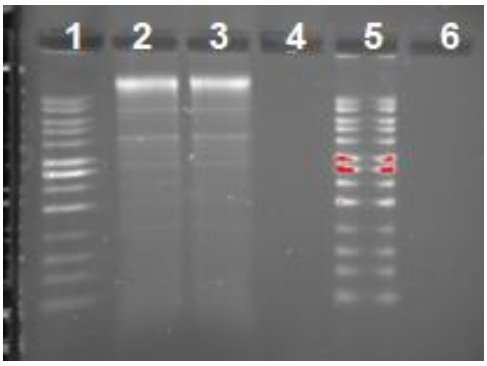


Figure 5. 200206 agarose gel of Q11 DNA digested by EcoRI.

Five tubes of EcoRI restriction enzyme digest of Q11 DNA were prepared. Each tube contained 30 µL of Q11 DNA, 4 µL of EcoRI buffer H, 0.4 µL BSA diluted to 10x in Promega Multi-core, 3.6 µL of sterile distilled water, and 2 µL of EcoRI restriction enzyme. Each sample was incubated at 37 °C for two hours before 10 µL of NEB Stop solution was added to two samples. The two samples containing NEB Stop solution were loaded onto an agarose gel in lanes two and three to confirm EcoRI digestion. Lanes one and five on the gel were loaded with 3 µL of 1 kb *Generuler* DNA ladder. Lanes four and six were left blank. The gel was run before being stained and imaged. The three remaining samples of EcoRI digested Q11 DNA were stored in a freezer at -20 °C for future use. These samples are referred to as EcoRI digests of Q11 DNA from 200206 in future experiments.

The digestion of Q11 DNA with EcoRI provided 9 visible bands on the agarose gel (Figure 5). These results match the previous results of EcoRI digestion of Q11 DNA on 200203. The Q11 DNA was not fully digested by EcoRI, a longer digestion time is recommended for the remaining samples of EcoRI digested Q11 DNA.

200213 EcoRI Restriction Enzyme Digestion Fragments of Q11 DNA treated with 5’ Exonuclease

Exonucleases, unlike restriction enzymes which cut DNA at specific recognition sites, completely digest double stranded DNA from the 5’ or 3’ terminus, unless something is bound to the end of the DNA, blocking activity of the exonuclease. If a 5’ terminal protein is covalently bound to the end of Q11’s DNA the terminal protein should block the exonuclease from digesting the protein, the DNA it is attached to.

A 5’ exonuclease (ThermoFisher) was added to EcoRI digested-Q11 DNA. Lane one was loaded with 5 µL of 1 kb *Generuler* DNA ladder. Lane two was loaded with 25 µL of a mixture containing 20 µL of Q11 DNA treated with EcoRI restriction enzyme from the 200206 experiment and 5 µL of NEB Stop solution. Lane three was loaded with 25 µL of a mixture containing 20 µL of EcoRI digested Q11 DNA, 2 µL of 5’ exonuclease, 3 µL of 5’ buffer and 5 µL of NEB Stop solution added to the solution after a two hour incubation at 37 °C.

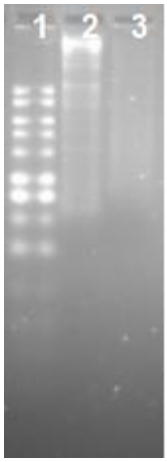


Figure 6. 200213 5' exonuclease and EcoRI digestion of Q11 DNA on agarose gel.

The EcoRI digestion of Q11 DNA produced most of the visible fragments shown in previous experiments 200203 and 200206. There were no visible fragments visible on the agarose gel after the 5’ exonuclease digestion of EcoRI digestion of Q11 DNA. A band should be visible on the gel if a 5’ terminally bound protein was covalently bound Q11 DNA. The absence of any bands after 5’ exonuclease digestion suggests that there is no protein bound to Q11 DNA. Further experiments will test fragments of Q11 DNA treated with EcoRI on SDS polyacrylamide gels, meant for protein separation. If a protein is bound to a Q11 DNA fragment it may migrate through the SDS polyacrylamide gel on the DNA fragment producing a band.

200214 SDS PAGE Gel of Q11 DNA Untreated and Treated with EcoRI

SDS polyacrylamide gel electrophoresis (SDS PAGE) was performed on whole Q11 DNA and Q11 DNA fragments treated with restriction enzyme EcoRI. SDS PAGE gels have smaller pores than agarose gels and are typically used for protein separation. These experiments were run with the idea that if a terminally bound protein exists it will migrate along with the DNA it is bound to and produce a band on the SDS-PAGE gel. Two gels were run for each SDS experiment to replicate results.

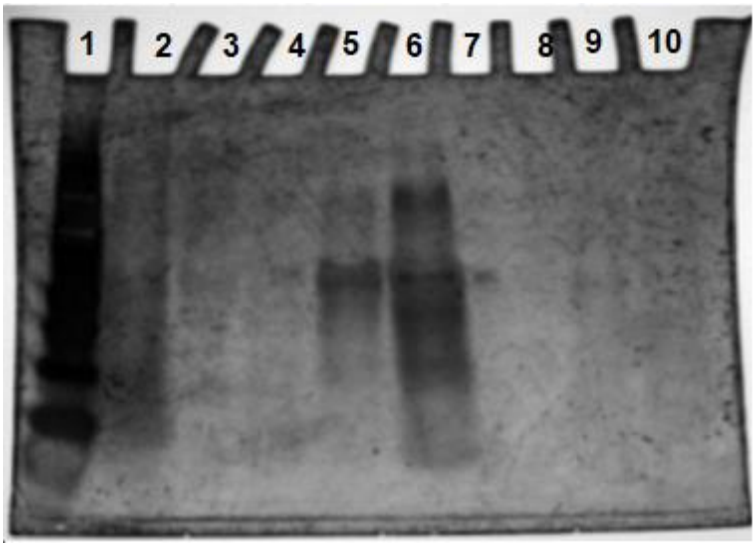


Figure 7. 200214 SDS PAGE gel 1 of Q11 DNA and EcoRI treated Q11 DNA. Silver Stain.

Lane 1 on gel 1 (Figure 7) was loaded with 5 µL of BioRad Protein-Precision-Plus Marker (PPP, BioRad, Hercules, CA). Lanes two, three and four were loaded with 5 µL each of Q11 DNA digested by restriction enzyme EcoRI on 200206 diluted to 0.1X, 0.01X, and 0.001X, respectively. Lane five was loaded with 5 µL of restriction enzyme EcoRI diluted to a 1/25 dilution in sterile distilled water so as to match the approximate amount of enzyme (protein) in the treated samples. Lane six was loaded with 1 µL

of undiluted restriction enzyme EcoRI. Lanes seven and eight were left blank. Lanes nine and ten were loaded with 5 µL each of untreated Q11 DNA diluted to 0.01X and 0.1X, respectively.

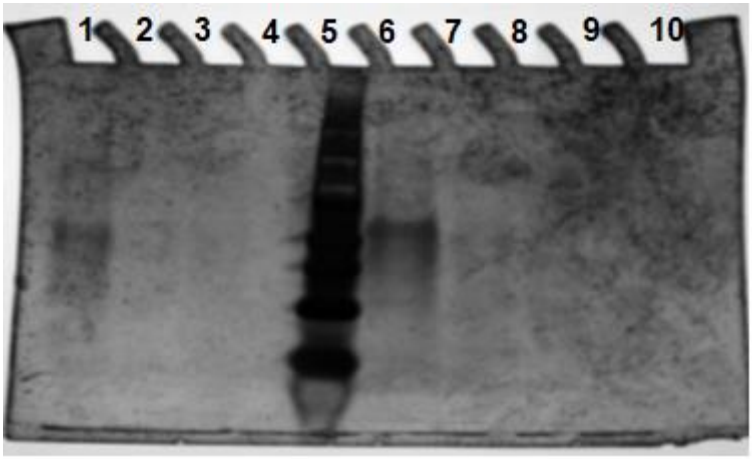


Figure 8. 200214 SDS PAGE gel 2 of Q11 DNA and EcoRI treated Q11 DNA. Silver Stain.

Lanes one, two, and three on Gel 2 (Figure 8) were loaded with 5 µL of Q11 DNA digested with EcoRI digest on 200206 diluted to 0.01X, 0.001X, and 0.0001X, respectively. Lane four was left blank. Lane five was loaded with 5 µL of PPP Marker. Lane six was loaded with restriction enzyme EcoRI diluted to a 1/100th dilution with sterile distilled water. Lanes seven, eight, and nine were loaded with 5 µL each of untreated Q11 DNA diluted to 0.01X, 0.001X, and 0.0001X respectively. Lane ten was left blank.

After loading, the SDS PAGE gel was run at 150 V for approximately three hours before being fixed, stained with Coomassie Blue, destained, Silver Stained and imaged using the methods described in the SDS PAGE methodology section. The results of this gel were surprising. Most DNA is presumed to be too large to migrate through an SDS gel, usually causing the gel to warp in the frame. However, every sample of DNA loaded into the gel appeared to migrate without causing warping of the gel. Most concentrations of DNA loaded were too faint to be seen on the silver stained image. On gel 2 (Figure 8), a faint potential band can be seen in lane seven where untreated Q11 DNA was loaded at a 0.01X concentration. A band in the presence of Q11 DNA could mean several things. The band appears in the most concentrated sample (0.01X) of Q11 DNA loaded onto this gel. The band could be a contaminant that is at a high enough concentration to produce a band on the gel. The band appears to be roughly the same size (similar migration distance) as the restriction enzyme EcoRI. The band could be spillover from loading EcoRI into the lane next to it. The absence of a band on gel one where untreated Q11 DNA was loaded at

the same 0.01X concentration appears to support the hypothesis that this band is a contaminant. Our hope is that this band is a protein fragment bound to Q11 DNA that migrated through the gel. The possibility of this band being migrated protein on the DNA will continue to be explored in future experiments.

This was also the first trial of an SDS gel run with Q11 DNA. Various concentrations of things like DNA, EcoRI, and marker will be adjusted in further experiments to produce a clean gel with bands of similar intensity when treated with silver stain.

200223 SDS PAGE Gel of Q11 DNA Untreated and Treated with EcoRI

Following the discovery of a band forming on gel 2 from the previous experiment (200214), the concentrations of marker and restriction enzyme EcoRI were lessened and more Q11 DNA, both untreated and treated with EcoRI from the 200206 experiment was used. The goal was to produce a gel that had consistent intensity between the marker, restriction enzyme and phage DNA when silver stained. More Q11 DNA at higher concentrations was used in an attempt to reproduce results from 200214 where a band was found on gel 2 well 7 (Figure 8) where Q11

DNA was loaded at a 0.01X concentration. Two polyacrylamide protein gels were run to replicate results within this experiment.

For gel 1 (Figure 9), lane one was loaded with 1 µL of marker at a 1x concentration. Lane two was loaded with 5 µL of marker at a 0.01X concentration. Lanes three and four were loaded with Q11 DNA treated with EcoRI from 200206, 5 µL at a 0.01X concentration and 10 µL at a 0.001X concentration, respectively. Lane five was loaded with 5 µL of restriction enzyme EcoRI at a 0.01X dilution. Lane six was left blank. Lanes seven and eight were loaded with 10 µL each of Q11 untreated DNA at a 0.1X and 0.01X concentration respectively. Lane nine was left blank. Lane ten was loaded with 10 µL of untreated Q11 DNA at a 0.1X dilution.

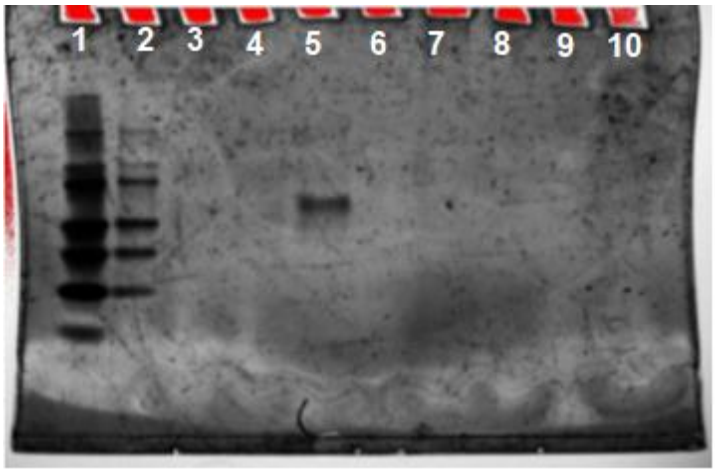


Figure 9. 200223 gel 1. SDS PAGE of Q11 untreated and EcoRI treated DNA. Silver Stain.

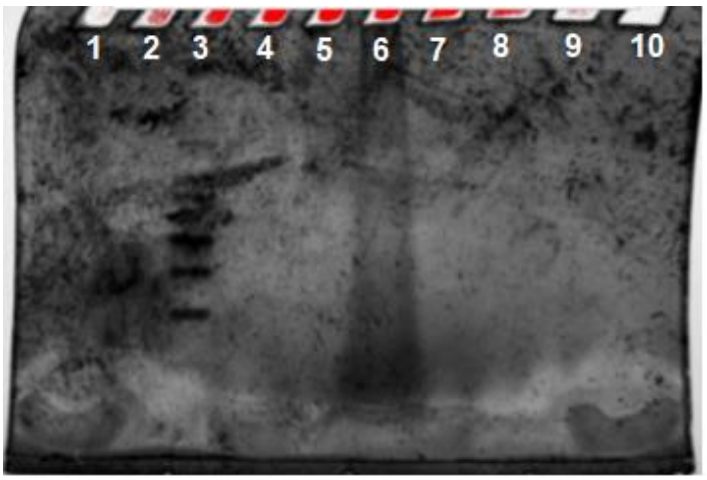


Figure 10. 200223 gel 2. SDS PAGE of Q11 untreated and EcoRI treated DNA. Silver Stain.

Gel 2 (Figure 10), lane one was loaded with 10 µL of 200206 EcoRI treated Q11 DNA at a 0.001X concentration. Lane two was left blank. Lane three was loaded with marker diluted to 0.01X. Lane four was loaded with restriction enzyme EcoRI at a 0.001X concentration. Lane five was left blank. Lanes six, seven, and eight were loaded with untreated Q11 DNA at a 1X, 0.1X, and 0.01X concentration respectively. Lane nine was left blank. Lane ten was loaded with 5 µL of untreated Q11 DNA.

All samples were treated as described above in the Materials and Methods section. The dilution of the marker in gel 1 lane two (Figure 9) produced clearer bands than loading a smaller

amount of 1X marker. The 0.01X dilution of restriction enzyme EcoRI on gel 1 (Figure 9) produced a band of similar intensity to the diluted marker in lane two after being silver stained. Untreated Q11 DNA at a 1X concentration on gel 2 (Figure 10) lane six was the only DNA band to produce something on the gel. The untreated Q11 DNA at 1X created a dark smear in its lane after being stained. There were no bands visible for Q11 DNA, treated with EcoRI or untreated at any concentration on the gel. Q11 DNA is surprisingly still able to migrate through the gel at every concentration. There was no visible warping of gel in any place including Q11 DNA at the 1X concentration. The grainy quality in both Figure 9 and 10 is due to the silver stain. All glassware involved in making up the silver stain solution will be rinsed with nitric acid to clean the glassware and reduce the grainy appearance of future gels.

200312 SDS PAGE Gel of Q11 DNA Untreated and Treated with EcoRI

This experiment is looking to either confirm or deny the existence of the band seen on gel 2 lane seven from the 200214 gel that was not seen at any Q11 DNA concentration on the 20223 gel. Two gels were run for this experiment to duplicate results.

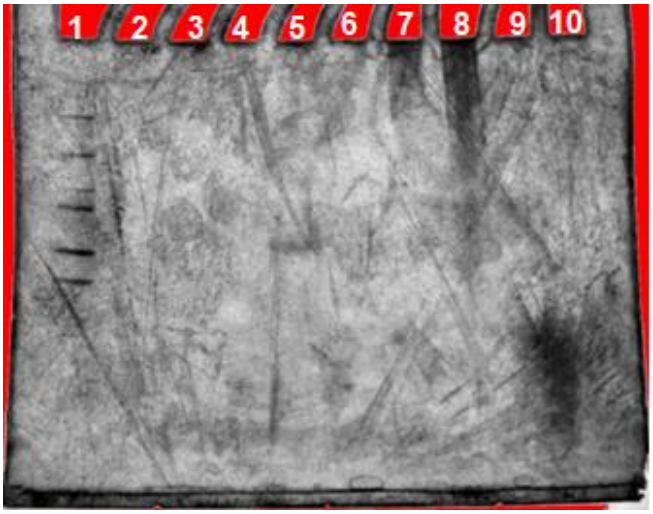


Figure 11. 200312 gel 1. SDS PAGE of Q11 untreated and EcoRI treated DNA. Silver Stain

Gel 1 lane one (Figure 11) was loaded with 5 µL of marker at a 0.01X concentration. Lanes two, three and four were loaded with 5, 10 and 15 µL, respectively, of 200206 EcoRI digested Q11 DNA. Lane five was loaded with 5 µL of restriction enzyme EcoRI at a 0.01X dilution. Lane six was left blank. Lane seven was loaded with 10 µL of untreated Q11 DNA at a 0.1X concentration. Lane eight was loaded with 5 µL of untreated

Q11 DNA at a 1X concentration. Lane nine was left blank. Lane ten was loaded with 10 µL of untreated Q11 DNA at a 0.01X concentration.

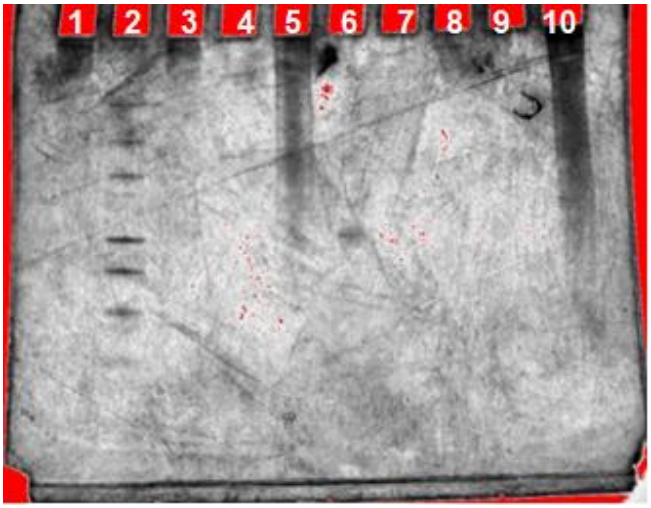


Figure 12. 200312 gel 2. SDS PAGE of Q11 untreated and EcoRI treated DNA. Silver Stain.

Gel 2 lane one (Figure 12) was loaded with 10 µL of untreated Q11 DNA at a 0.1X concentration. Lane 2 was loaded with 5 µL of marker at a 0.01X concentration. Lane three was loaded with 20 µL of untreated Q11 DNA at a 0.1X concentration. Lane four was left blank. 5 µL of untreated Q11 DNA at a 1X concentration. Lane six was loaded with 5 µL of restriction enzyme EcoRI at a 0.01X dilution. Lanes seven and eight were loaded with 10 and 20 µL respectively of untreated Q11 DNA at a 0.01X concentration.

Similar to the 200223 experiment, no discernable DNA band was visible on the gel. Both samples of restriction enzyme EcoRI produced a band, as well as the marker proteins loaded in each gel. Untreated Q11 DNA loaded into the gel at a concentration at or above 0.01X produces a smear that is stained in the lanes where the untreated DNA is loaded. After multiple attempts to observe a band on the gel for the suspected Q11 protein, no band similar to the one observed on 200214 has been found.

Discussion

Conclusions

The absence of bands on the 5’ exonuclease (ThermoFisher) and EcoRI restriction enzyme treated sample indicate that terminally bound protein may not exist on Q11’s DNA. Based on previous studies of Phi29, which has a confirmed terminal protein, treatment with a 5’

exonuclease does produce fragments when run on a gel. The absence of results in our experiment is worth another run of 5' exonuclease λ digestion to confirm these results.

The presence of a band in the 200214 SDS-PAGE gel image where untreated Q11 DNA at a 0.01X concentration was loaded, suggested that there may be a terminally bound protein that migrated with the DNA it is attached to. Several other trials 200223 and 200312 were conducted in an attempt to replicate the results seen on the 200214 gel. No concentration of Q11 DNA treated with EcoRI or untreated was able to produce a band similar to what was observed on the 200214 gel. It can be concluded that the most likely cause of the band observed on the 200214 gel was either a contaminant or spillover from loading restriction enzyme EcoRI in the neighboring well.

This research demonstrated there is not enough conclusive evidence to prove or disprove the existence of a terminal protein covalently bound to the end of Q11's DNA. The results of this experiment suggest that a 5' terminally bound protein may not exist despite earlier experiments[3,4,9] suggesting that a 5' terminal protein does exist for Q11. Further investigation is required to pursue the idea that a protein may be bound to Q11 DNA.

Areas for further investigation

This research project was not able to conclusively prove that a protein is covalently bound to the end of Q11's 5' DNA. While this research project is ongoing, the preliminary results presented in this paper cannot conclude that a covalently bound protein is present on the 5' terminal end of bacteriophage Q11's DNA. Continued research into this topic will build upon the methods and techniques in this paper to determine the existence of a covalently bound protein.

If Q11 can be shown to have a covalently bound protein on the end of its DNA, further investigations will be conducted to isolate, replicate and determine the structure and function of the protein in relation to Q11's virulence and survival in the environment and inside of a bacterial

host. Several other options could be considered for something that blocks DNA sequencing of Q11 on the terminal end. If no covalently bound protein can be found on the end Q11's DNA further investigation will be taken to understand why Q11 DNA was previously unable to be sequenced in previous experiments.[3]

The understanding of bacteriophages that can kill and infect bacterial species is critical for both a more developed scientific understanding and as an emerging healthcare tool to treat bacterial infections. Every drug that passes inspection through the Food and Drug Administration (FDA) must be studied and researched carefully before the product is released onto the market for patient treatment. The same is absolutely true, if not more so for viruses as a potential treatment for bacterial infections. This is a type of biotechnology that has been historically developed but is only recently seeing use against human bacterial infections. Our current healthcare system has relied on antibiotics to treat bacterial infections for almost a century. Bacteria may lack complexity but compensate at the rate at which they are constantly dividing and growing. Constant treatment of bacterial infections with a limited number of antibiotics has led to bacterial resistance to the antibiotics that we rely on to kill them. Novel methods of treating bacterial infections need to be explored.

Bacteriophages are viruses that naturally infect and kill bacteria. These phages replicate at a much faster rate than bacteria, potentially only requiring a small dose of phage that will rapidly replicate in the body to fight a bacterial infection. Because viruses have a much quicker replication cycle than bacteria, they can constantly evolve to evade bacterial defenses to ward off infection, like they have been doing for millions of years. It makes sense, as we move into a new era of biotechnology, to consider using phages that have evolutionarily evolved over hundreds of millions of years to infect and kill bacteria as a novel treatment when antibiotics no longer work. Research into novel medical treatments takes time. Years of work need to be put into understanding these viruses before they are able to be tested for medical purposes. Every aspect

of these viruses needs to be understood down to the genetic information that encodes and evolves for their survival. It is thought that terminal proteins in certain viruses aid in the replication of necessary proteins for the survival and infectivity of the virus. Understanding these components allows researchers to get one step closer to applying the knowledge we can learn in the lab to a real-life medical application that has the potential to save lives.

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